NON-DIAPAUSE OVERWINTERING BY *PIERIS RAPAE* (LEPIDOPTERA: PIERIDAE) AND *PAPILIO ZELICAON* (LEPIDOPTERA: PAPILIONIDAE) IN CALIFORNIA: ADAPTIVENESS OF TYPE III DIAPAUSE-INDUCTION CURVES*

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Diapause is generally regarded as a physiological adaptation which increases the probability of surviving the adverse season, and thus of reproducing after it is over. Many insect species show geographic differences in the environmental regimes which induce or inhibit diapause (e.g., critical photoperiod) and in the strength of the diapause induced. Such interpopulational differences are commonly viewed as "fine tuning" to local climates, accomplished by natural selection and reflecting a genetic basis (e.g., Istock, 1981). Intrapopulational differences in photoperiodic sensitivity and diapause strength (e.g., chilling requirement) also occur, and have been interpreted as polymorphisms which "spread the risk" of environmental uncertainty over the population (cf. Bradshaw 1973, Shapiro 1979, 1980a). In multivoltine insects in seasonal climates, offspring produced by the last seasonal generation of adults are commonly induced to enter diapause by specific combinations of environmental factors; in mid-latitudes these are likely to be decreasing photophase/increasing scotophase and decreasing or consistently low night temperatures. Warmer nights tend to shorten the critical photoperiod for a given population, or may effectively inhibit diapause altogether under field conditions.

Beck (1980) characterized diapause induction as falling into four response types. Type I is the common long-day response, in which long days inhibit diapause and there is a single threshold beyond which diapause occurs. Type II, the short-day response, is the reciprocal of Type I. Type III has two well-defined critical day-lengths, such that diapause is induced between, say, 8 and 14 hours

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light, and inhibited when either less than 8 or more than 14 hr of light occur. Type IV is its reciprocal, in which diapause is inhibited over a relatively narrow range and induced both above and below. Of the four types, Type I is by far the commonest in temperate-latitude insects and Type IV the rarest. Low-latitude insects have rarely been examined for photoperiodic responses, though the different patterns of seasonality in low latitudes might be expected to produce a rather different picture than that seen in temperate zones.

Type III curves are known from a variety of insects. Among Lepidopterans, two important species that show them are the European Corn Borer, *Ostrinia nubilalis* (Hbn.) (Beck, 1962) and the Large White, *Pieris brassicae* L. (Danilevskii, 1961). The adaptive significance of the short-day threshold is unclear. Normally the entire population would have been induced by day-length-temperature interaction to enter diapause before the inhibition threshold was reached. Thus, the photosensitive stages would never be exposed to such short photoperiods in nature, and the existence of a short-day threshold would seem nonadaptive. This paper reports a situation in which Type III curves were demonstrably adaptive for two multivoltine Lepidopterans in nature.

The Systems

Papilio zelicaon Lucas and *Pieris rapae* L. are very common, widespread multivoltine butterflies in lowland California. Both have been monitored phenologically for up to 13 yr along a transect parallel to Interstate Highway 80 from sea level at the Suisun Marsh, Solano County, to treeline in the Sierra Nevada. We are concerned here with the phenology of populations at low elevations between the San Francisco Bay area and Sacramento. The flight seasons for both species may reach ten months (Table 1), with four (*P. zelicaon*) to about six (*P. rapae*) generations per year. Both species have facultative pupal diapause under conventional photoperiod-temperature control (Sims, 1980; Shapiro, unpublished data); both have Type III diapause-induction curves. Diapause is irreversibly determined no later than the fourth instar. *P. zelicaon* is the stronger diapauser and is unrecorded at any of our sampling sites between 1 December and 18 February. In some years the period with no *rapae* flying may, however, be as short as four weeks.

Table 1. Dates of first and last observed flights of two common multivoltine butterflies in lowland north-central California, based on standardized methodology and level of effort.

	Pieris rapae				*Papilio zelicaon*		
	Davis-Sacramento area[1]		Suisun Marsh[2]		Davis-Sacramento[1]	Suisun Marsh[2]	
	First	Last	First	Last	First[3]	First	Last
1972	ii.26	xi.29	--	x.21	iii.5	--	xi.5
1973	i.20	xii.24	--	xii.29	iii.14	iii.18	xi.18
1974	ii.1	xii.15	ii.2	xi.25	iii.19	ii.23	x.26
1975	ii.16	xii.5	i.30	xi.13	iv.11	iii.5	x.15
1976	i.25	xii.20	ii.15	xi.21	iii.10	iii.9	x.16
1977	--	xii.23	--	x.31	iii.6	iii.5	x.14
1978	ii.18	xii.2	ii.14	xi.15	iii.18	iii.10	x.11
1979	ii.17	xi.9	iii.5	xii.1	iii.31	iii.23	x.7
1980	ii.10	xi.26	--	xi.23	iii.28	iii.9	ix.28
1981	ii.14	xii.16	ii.21	xi.7	ii.22	iii.10	x.18
1982	ii.2	xii.19	ii.17	x.23	iii.20	iii.19	x.23
1983	ii.13	xii.18	iii.4	xii.17	iii.11	iii.14	x.23
1984	i.28		i.29		iii.6	iii.4	

Notes: [1]Consists of several study sites in Yolo and Sacramento Counties, all below 40m elevation.
[2]Near Fairfield, Solano County, at sea level.
[3]Last flight data not given for inland populations because numbers are usually very low late in the season as compared to near-coastal populations.

In contrast, the Pierid *Colias eurytheme* Bdv., which overwinters as a quiescent (non-diapausing) larva, flew continuously during the drought years of 1975–76 and 1976–77 and nearly so in 1983–84. Collections of mature larvae of both *P. zelicaon* and *P. rapae* made in late October and November over 12 yr have consistently given from 85–100% diapause pupae. Larvae of *P. zelicaon* are normally absent from all sites by the third week of November. *Rapae* larvae are occasionally found on garden cabbages and weedy Crucifers into early January. A fifth-instar larva was collected on a weed on 29 January 1979.

The winter of 1982–83 produced 200% or more of normal rainfall over most of northern and central California. Nearly 20 cm of rain fell in March 1983 at our study sites, and the weather remained showery and unsettled into May. The spring flights of most butterflies were late and poor; for many species the densities observed were the lowest in 12 yr. Both *P. rapae* and *P. zelicaon* were severely depressed at most sites; *P. rapae* was actually rare in spring, and the Willow Slough population of *P. zelicaon,* north of Davis, went extinct overwinter. The summer was unusually cool, cloudy, and moist. By autumn the populations of both species were near normal levels and at the Suisun Marsh *P. zelicaon* was commoner than usual at the end of the season. The latest flight recorded at Suisun for this species is 18 November (1973), a "false brood." Although the species shut down nearly a month earlier in 1983, the autumn brood oviposited three to four weeks later than average. Similarly, at Davis, the last flight date of *P. rapae* was unexceptional but the population densities at the end of the flight were unusually high. For both species, these circumstances translated into an unprecedented number of larvae through mid-winter.

In the Suisun Marsh, fourth- and fifth-instar larvae of *P. zelicaon* were common on Sweet Fennel, *Foeniculum vulgare* Mill. (Umbelliferae) on levees; collections were made on 28 December (4 L_5, 1 L_4, 1 L_3) and 16 January (2 L_5). In addition, one L_3 was found on the same plant at Gates Canyon in the Vaca Hills, Inner Coast Range, near Vacaville, Solano County, 1 January.

In the Davis area, a total of 67 larvae of *P. rapae* were collected from cultivated and weedy Cruciferae and *Tropaeolum* between 29 December and 15 January. Although L_1–L_3 were still numerous early in the period, only larger larvae (L_4–L_5) were taken. Numbers were so high that Cruciferous vegetables were seriously damaged in many gardens at this time.

All larvae were placed in outdoor mesh cages over fresh cuttings of their hosts, and allowed to complete development under ambient conditions. The cages were less than 0.3 km from the U.C. Davis campus meteorological station. The first pupa of *P. zelicaon* was formed 13 January and the last 24 February. 50 of the 67 *rapae* larvae were parasitized by *Apanteles (Cotesia) glomeratus* (L.) (Hymenoptera: Braconidae) and failed to pupate; 5 died of unknown causes; 12 pupated between 9 January and 28 February.

Results and Discussion

Of the nine winter *zelicaon,* 1 prepupa died of unknown causes; 5 eclosed without diapause; and 3 apparently entered diapause. Eclosions occurred on 21 and 24 February and 1, 5, and 12 March 1984. As can be seen from Table 1, these dates coincide with the flight of *P. zelicaon* in the field. (At Gates Canyon, Vaca Hills, one *zelicaon* was found as early as 19 February 1984, the earliest flight ever recorded in the region.) All the butterflies which emerged were female, as was one of the 3 diapausers.

Of the 12 healthy *rapae* pupae, all eclosed between 9 February and 24 March (seven males, five females). The flight began somewhat earlier afield but continued throughout this period.

The *Apanteles* wasps emerged without diapause in synchronized batches, each from its own host, between 24 January and 11 March—again, well-timed to parasitize first-instar larvae from eggs laid by first-brood *rapae* females. Diapause in this species is known to be under direct photoperiodic control (rather than mediated through the host, hormonally) in the U.S.S.R., but has not been studied in the introduced North American populations (Danilevskii, 1961).

The phenotypes of emerging adults of both butterflies were compared with long series of field-collected specimens from Suisun and Davis from 1984 and prior years, including many individuals which must have come from diapaused pupae, and with reared ex-diapause individuals. No phenotypic differences which might permit the detection of non-diapaused adults in the spring populations were recognized. This result confirms experimental results which indicated that the post-diapause adult phenotype is not physiologically coupled to the prior developmental arrest, but is rather a function of ambient conditions after reactivation (Shapiro, 1975a, 1978), superimposed on irreversible short-day prediapause determination.

As is evident from Table 2, low-temperature lethality is a rare event in this part of California. Although diapause may confer a degree of frost-tolerance, its principal benefit in *P. zelicaon* and *P. rapae* in north-central lowland California seems to be to delay the onset of adult development until the bulk of the rainy season has passed; thus, adult eclosion coincides with sunny, warm weather suitable for flight and hence for reproduction. Pupae accumulate enough chilling by roughly mid-January to come out of diapause; subsequent development is timed by the weather, which thus determines not only the date of first flight but the degree of synchronization of the spring brood (Shapiro, unpublished data). The unusual 1983–84 winter larvae pupated at about the same time as normal diapausing pupae would have resumed development; hence it is not surprising that their subsequent development was synchronized with the wild population. (On the other hand, diapaused pupae of *Pieris napi* L. ssp. will develop to the pharate adult at a constant temperature of 3° C, while non-diapause ones will not; hence in that species, diapausers might be expected to eclose first; Shapiro, unpublished.)

The 3 diapausing *zelicaon* presumably cannot accumulate enough chilling to break diapause in spring 1984 and will thus lay over until early 1985. This would expose them to additional risks of mortality, and in terms of contribution to the rate of population increase, place them at a great selective disadvantage relative to nondiapausing members of their 1983–84 cohort (a delay of 4 generations). Multiple-year diapause occurs in most populations of *P. zelicaon* even under normal circumstances, although it is rare in the multivoltine populations; it is especially common in univoltine foothill races which face unusually unpredictable and stressful climates and which would be expected to engage in "risk-spreading" (Sims, 1980). On the other hand, although pupae of *P. rapae* will remain viable for two years under constant refrigeration, no multiple-year diapause has ever been observed in that species under field conditions, and there is no indication that it has any physiological ability to diapause in summer (Shapiro 1975b, 1980b). Thus any diapause pupae produced by winter larvae of this species would presumably be doomed.

In a Mediterranean climate, then, a Type III diapause-induction curve permits stragglers at the end of the season to complete development and enter the reproductive pool the following spring at

Table 2. Climatic parameters for Sacramento (from U.S. Weather Bureau, NOAA Technical Memorandum NWS-WR-65, 1971). Temperatures are in degrees Fahrenheit. This table covers the period from the beginning of the last autumn generations of *P. rapae* and *P. zelicaon* to the end of the diapause in spring.

			Temperature (To 1971)				
Date	Sunrise (PST)	Sunset (PST)	daily mean	mean max	mean min	record high	record low[1]
Sept. 15	0547	1815	72	87	56	104	47
Oct. 1	0602	1749	69	83	54	98	43
Oct. 15	0615	1728	64	77	51	94	38
Nov. 1	0632	1706	59	70	47	86	34
Nov. 15	0648	1653	55	66	43	80	29
Dec. 1	0705	1645	50	59	40	71	32
Dec. 15	0716	1646	47	55	39	72	26
Jan. 1	0724	1656	46	53	39	65	24
Jan. 15	0722	1709	46	54	38	67	19
Feb. 1	0712	1728	48	57	39	68	28

Notes: [1]Low lethal temperature for *P. rapae* pupae not in diapause is ca. 22° F; for *P. zelicaon* ca. 19° F. Not determined for larvae, but both species can cool at least to the mid-20s overnight in protected micro-sites.

no disadvantage; it is a valuable hedge against off-season reproduction. In such benign climates the photoperiodic threshold for diapause inhibition may be under intermittent selection at both ends of the curve. Non-diapause overwintering may be fairly frequent in some California butterflies, especially *P. rapae.* At the latitude of Sacramento it is definitely rare in *P. zelicaon,* since midwinter larvae were not observed in the first 12 yr of this study. In coastal southern California, however, this species flies more or less all winter (Emmel and Emmel, 1973; Orsak, 1977; Shapiro, unpublished) and larvae may often be found during the shortest days of the year. Although diapause intensity is reduced and thresholds are moderately shifted relative to further north, even the San Diego populations retain the ability to diapause (Sims, 1980). How often they use it under field conditions may depend on the timing of autumn reproduction on a year-by-year basis. The hypothesis that both thresholds of a Type III curve are adaptive and under selection is testable in principle by examining latitudinal shifts in critical photoperiods.

We have had under study a culture of *P. rapae* from Xochimilco, D.F., Mexico, the southernmost (ca. 19°N) population of this species in the Americas; it also displays a Type III curve with the short-day inhibition threshold shifted upward (Shapiro, in preparation).

In climates where the onset of cold is more rapid than the change in photoperiod, that is, where strong air-mass contrasts exist and thermal lethality can occur with great suddenness, it is difficult to envision the low end of a Type III curve as adaptive. Cold nights should assure conservatism in the critical photoperiod, so that nearly all individuals will be determined as diapausers before the inhibition threshold can be reached. Larvae of *P. rapae* can be found into December and rarely into January at Philadelphia and New York City as well when early winter conditions are mild, but they never seem to survive. On the other hand, the occasional very early onset of lethal cold in such areas should select for more conservative diapause induction than the average conditions seem to warrant.

The natural selection of diapause characteristics, already complicated by its nature as a recurrent (cyclical) process operating every 4th and 5th generation, is further complicated by the ability of stochastic variation to override it occasionally in benign climates.

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